of KCN that the Heinz body formation was inhibited in red cells containing methemoglobin, as compared with control series. A typical experiment is presented in the Figure.

The question is: how the methemoglobin inhibits the Heinz body formation? The cause of the inhibition may be that the methemoglobin binding all the KCN, the breakdown of hydrogen peroxide is caused by the uninhibited catalase instead of peroxidatic utilization. This possibility was also examined. 0·1 ml KCN-blood mixture was added to 10 ml of $M/60$ phosphate buffer, pH 6·6, and the optical density was determined at 635 mμ before and after adding a drop of neutralised sodium cyanide. There has been no decrease of the optical density in the first (3×10^{-1} M KCN) and in the second sample (3×10^{-2} M KCN). It seems certain that all the ferric ions of the methemoglobin molecules, and likely that all the ferric ions of the catalase molecules, were bound to the cyanide in the second sample and there must be some excess cyanide in the first sample. Another possibility is that the methemoglobin, binding all the hydrogen peroxide, causes a hydrogen peroxide deprivation for the catalase and in this way inhibits the peroxidatic function of catalase.

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Zusammenfassung

Die methämoglobinisierten (mit Nitrit vorbehandelten) roten Blutkörperchen sind *in vitro* der Heinz'sche Körperchen bildenden Wirkung des Zyanid-Peroxyd-Testes gegenüber resistenter als normale Erythrozyten.

Autoradiographs of Human Sera Tagged with Radioactive Thyroxine and Investigated in Immunoelectrophoresis

The thyroxine circulating in the blood is mainly bound to proteins. It has been found that an α-globulin is likely to be the physiological carrier of the hormone[1–3]. This globulin has been referred to as the thyroxine-binding protein (TBP). If the blood concentration of thyroxine is increased, there is also a loose attachment to the albumin. Titration of the unutilized binding capacity of TBP can therefore be done by adding labelled thyroxine to plasma, using the albumin as a reference[2,3]. Normally, one third of the binding capacity is utilized[4]. Further studies of TBP have revealed that it has a molecular weight of about 50000, an iso-electric point below pH 4·5, and that it seems to be a glycoprotein[5,6]. Since thyroxine is relatively loosely attached even to the TBP, further characterization of the TBP has not been determined.

In contrast with ordinary electrophoretic methods, by means of immunoelectrophoresis[7] it is possible to obtain a closer characterization of precipitating components of, for example, human sera. Therefore, radioactive synthetic thyroxine[8] was added in varying amounts to plasma of a healthy euthyroid adult male. Pairs of samples of the plasma were then separated by means of agar-gel electrophoresis on object slides[9]. One of them was fixed in acetic acid, dried, and autoradiographed. On the other slide, the electrophoretically separated components were allowed to diffuse against a rabbit anti-human serum[7,9,10]. This slide was then dried and autoradiographed. The development revealed arc-shaped lines, the position of which were identical with certain precipitates in the agar-gel (Fig.). Two radioactive spots were obtained in the agar-gel electrophoretic pattern and two distinct arcs appeared in the radioautograph of the other slide when the concentration of the added thyroxine did not exceed 1 μg/ml plasma. The corresponding precipitates have been tentatively characterized as the α_1-glycoprotein[11] and the albumin. A higher concentration of labelled thyroid hormone gave a diffuse pattern in the agar-gel radioautogram but not any new arcs.

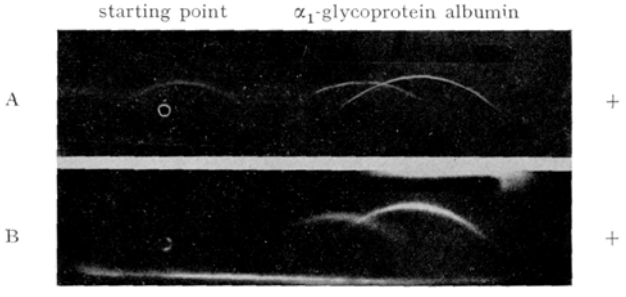

Fig. A: Immuno-electrophoretic pattern of a sample of human plasma to which labelled thyroxine was added (1 μg/ml). Starting point, the position of the α_1-glycoprotein and the albumin precipitates as indicated at the top. Negative pole was on the left, positive on the right. Slight retuch of the α_1-glycoprotein arc. On the original slight at least 14 precipitates were seen

Fig. B: Radioautograph of the plate shown in A. Although the radioactivity of the α_1-glycoprotein precipitate was weaker than that of albumin arc, the specific activity of the former was higher because the glycoprotein was present in the plasma in much lower concentration than the albumin

The finding of distinct radioactive precipitates from plasma to which small amounts of labelled thyroxine had been added, permits the following conclusions. It is highly probable that the TBP is at least partly identical with an α_1-glycoprotein; this is in agreement with previous assumptions in the literature[3,5,6]. We cannot exclude the existence of other TBP components not developed by the immune serum used, but they must occupy closely identical electrophoretic positions. No radioactivity could be

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demonstrated in the precipitates of the α_1-lipoprotein, α_1-seromucoid, and a component designed as the 'α_1-globulin I' which hitherto are the only components found in the near neighbourhood of the α_1-glycoprotein under the present experimental conditions[12]. It is of interest to note that precipitate formation does take place even in the presence of an excess of thyroxine.

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Zusammenfassung

Radioautographien menschlichen Plasmas, dem kleine Mengen von radioaktivem Thyroxin zugesetzt worden war, zeigten ein Präzipitat bei der Immunoelektrophorese mit Characteristika, die denen des α_1-Glykoproteins entsprechen. Grosse Mengen des Thyroxins verbanden sich auch mit der Albuminfraktion.

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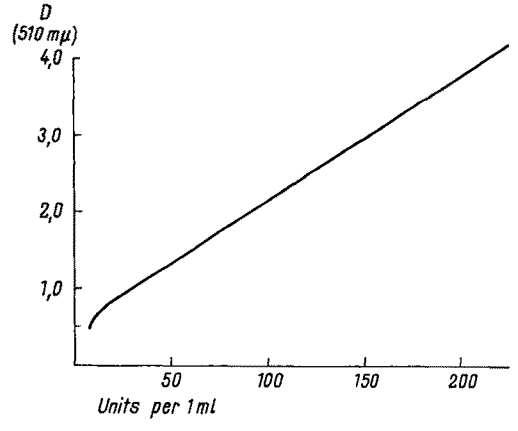
Transaminase in Seminal Plasma of Man

Several enzymes have been detected in the tissues of male accessory organs of reproduction (phosphatases, 5-nucleotidase, ATP-ase, aldolase) (MANN[1]). As far as transaminases are concerned, the prostatic gland and seminal vesicles are shown to be rich in these enzymes (BARRON and HUGGINS[2], AWAPARA[3]). However, the glutamic oxaloacetic transaminase (GO-T) has not yet been reported in the whole semen or in the seminal plasma.

Studying the GO-T activity of several human secretions and body fluids, we were impressed by the high values obtained in seminal plasma (POVOA and VILLELA[4]).

The high content of certain free amino acids and of citric acid in this fluid is possibly due to the transamination of glutamic acid and production of oxaloacetic acid. AWAPARA[3] pointed out that, in the prostatic gland, transamination is the only way of desamination.

Materials and methods: The GO-T was determined by an adaptation of the method of REITMAN and FRANKEL[5] used for blood serum. A calibration curve was constructed



according to these authors, employing 0·05 ml instead of 0·2 ml. The readings in optical densities at 510 mμ were plotted against units per 1 ml of seminal plasma as seen in the Figure.

Whole semen was centrifuged and the clear seminal plasma pipetted off. All the semens were previously submitted to standard tests so as to establish a criterium for normality (volume, shape, number, vitality, and motility of the spermatozoa).

Plasma in the volume of 0·05 ml was incubated 60 min at 37°C with 0·5 ml of the substrate (aspartate -α-ketoglutarate, pH 7·5) added with 0·5 ml of 2,4-dinitrophenylhydrazine hydrochloride 10^{-3} *M* and the colour developed after 20 min by adding 5 ml of 0·4 *N* NaOH. The optical density was read at 510 mμ in a spectrophotometer after 30 min against a blank. The values using this method are in accordance with those obtained with the method of KARMEN, WROBLEWSKI, and LADUE[6].

Results: The results obtained with the seminal plasma of normal, oligospermic, and azoospermic cases are presented in the Table.

Human seminal plasma Glutamic-oxaloacetic transaminase

Condition	No. of cases	No. spermatozoa 10^6/1ml	GO-T [a] Range	GO-T [a] Average and S.D.	t	P
Normal . . .	10	41–131	150–600	365 ± 170	—	—
Oligospermia	10	2–25	130–460	271 ± 110	1·4	<0·05
Azospermia .	5	0	80–260	172 ± 78	3·0	<0·01

[a] Values expressed in Reitman-Frankel units/ml

Normal seminal plasma showed values (average 365 ± 170 units) twenty times the normal blood serum (average 18 units). A marked decrease was observed in oligospermia and chiefly in azoospermia. The decrease for azoospermia was statistically significant ($t = 3{\cdot}0$; $P < 0{\cdot}01$) and for oligospermia less significant ($P < 0{\cdot}1$).

Since the enzyme activity is still elevated in the absence of spermatozoa, it seems that the conditions of the prostatic and seminal glands must be responsible for the low values found.

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Biochemical Laboratory, Instituto Oswaldo Cruz, Rio de Janeiro (Brazil), October 9, 1959.

Résumé

La transaminase glutamo-oxaloacetique (GO-T) a été déterminée dans le liquide spermatique de l'homme après la séparation des spermatozoïdes. On a trouvé des valeurs élevées pour la GO-T dans le sperme normal et une diminution accentuée dans les cas d'oligospermie et surtout d'azoospermie. Les conditions de la prostate et des vésicules séminales semblent être responsables de cet abaissement de l'activité enzymatique puisque le liquide sans spermatozoïdes (azoospermie) présente encore une activité bien marquée.

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